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Bioactive amines in fresh, canned and dried sweet corn, embryo and endosperm and germinated corn

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ABSTRACT

The types and levels of free bioactive amines in different corn products were determined. The amines were analyzed by ion pair-HPLC, post-column derivatization with *o*-phthalaldehyde and fluorimetric detection. Fresh sweet corn contained mainly spermidine followed by putrescine. Spermine, cadaverine, phenylethylamine, histamine and agmatine were also present at low levels. The profile and levels of amines changed significantly in canned and dried corn. Putrescine was the prevalent amine in canned corn whereas spermine was prevalent in dried corn. Germinated corn had significantly higher spermidine, spermine and putrescine levels. The embryo of the corn contained significantly higher spermine levels compared to the endosperm. These results indicate that corn is a good source of polyamines and that the different types of corn products available can be used to provide a profile of amines according to specific dietary need.

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1. Introduction

Maize (*Zea mays* L.), commonly known as corn, is a cereal widely used in human food and animal feed. The world production is 793 million tonne, whereas 52 million tonne is produced in Brazil. Of the total Brazilian production, 30% is used for human consumption with a large diversity of products available in the market. The most commonly used corn products in Brazil are sweet corn either fresh or canned and dried grain, available as different types of flour (IBGE, 2010; USDA, 2010).

Corn can supply several nutrients and energy in the diet. In addition, corn is considered to be a good source of polyamines, which are part of a larger group of biologically active substances, called bioactive amines (Gloria, 2005). The polyamines spermidine and spermine are essential for living cells, playing important roles in various physiological functions (Kalač & Krausová, 2005; Valero, Martinez-Romero, & Serrano, 2002). They modulate and promote growth (Bardócz, 1995). They are involved in the synthesis of DNA, RNA and protein and in the stabilization of cell membranes (Moinard, Cynober, & Bandt, 2005). They also promote the renewal and functionality of the digestive tract and maturation of the intestinal mucosa (Bardócz, 1995; Janicka-Russak, Kabala, Młodzinska, & Klobus, 2010; Moinard et al., 2005). Furthermore, they have antioxidant and anti-inflammatory properties (Gaboriau, Vaultier, Moulinoux, & Delcros, 2005; Løvaas & Carlin, 1991).

Corn can also be a source of biogenic amines. Some biogenic amines can be naturally present in corn whereas others can be

introduced during production, processing and storage. They can be formed by thermal or microbial decarboxylation of amino acids and may be used as an index of quality or hygienic conditions of products. These amines, at low concentrations, can play important roles in growth and protection of plants against predators and environmental factors. In the diet of animals, these amines can act as vaso- and neuro-active substances; however, at high concentrations some amines can be hazardous to human health (Bardócz, 1995; Gloria, 2005).

Therefore, the presence of corn in the diet can be advantageous due to the several functional and health promoting properties associated with polyamines and other amines. However, little information is available regarding the types and levels of amines in the different corn products available in the market.

Recently, the consumption of germinated or sprouted corn (from seed germination) is becoming popular. Germinated corn and its flour have been widely used for breads, some types of pasta and also beer brewing (Arasaratnam, Mylvaganam, & Balasubramaniam, 1998; Frías et al., 2007). Germination is the practice of soaking and draining the seeds until they germinate. It is a very efficient way to increase the nutritive value and the digestibility of seeds (Modgil, Joshi, Gupta, Verma, & Anand, 2009). During germination, natural starches are converted into digestible and simple sugars. Therefore, breads, flours, and pastas produced with sprouted grains are more digestible. Furthermore, they contain higher levels of carotenes, B vitamins and enzymes. The germination process can also remove naturally occurring toxins (Prodanov, Sierra, & Vidal-Valverde, 1997).

According to Gloria, Tavares-Neto, Labanca, and Carvalho (2005), germinated vegetables can contain higher levels of

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polyamines due to the rapid cell proliferation during the early stages of growth. During germination, there can also be formation of biogenic amines due to the physiological changes in the tissues and/or due to the activity of bacterial decarboxylating enzymes. The warm and moist environment is conducive to rapid proliferation of microorganisms including *Enterobacteriaceae* and *Pseudomonas spp.*, known to produce amino acid decarboxylases (Gloria, 2005).

Although all cells are capable of producing polyamines, there are some instances when higher amounts are required. Therefore, a continuous supply of polyamines from the diet is required (Bardócz, 1995). The objective of this study was to determine the profile and the levels of polyamines and other bioactive amines in corn products commonly available in the Brazilian diet, including germinated corn which is becoming popular worldwide.

2. Materials and methods

2.1. Samples

Corn products were purchased from the market of Belo Horizonte, MG, Brazil. The samples included: fresh sweet corn from the cob, canned sweet corn and dried corn. Canned sweet corn was used to obtain two separate products: embryo and endosperm. Germinated corn was produced using two corn cultivars (BRS2020 and PL8080), which were provided by a seed Producers Association of Minas Gerais, Belo Horizonte, MG, Brazil. Germination was accomplished by keeping the seeds in an incubator at $22 \pm 2^\circ\text{C}$, $90 \pm 2\%$ relative humidity and in the presence of light. They were analyzed before and at the 5th germination day. At least three different lots of each product were analyzed in triplicate.

2.2. Chemicals

Bioactive amine standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). They included spermine tetrahydrochloride, spermidine trihydrochloride, putrescine dihydrochloride, agmatine sulphate, cadaverine dihydrochloride, 5-hydroxytryptamine (serotonin), histamine dihydrochloride, tyramine hydrochloride, 2-phenylethylamine hydrochloride and tryptamine. *o*-Phthalaldehyde was also purchased from Sigma Chemical Co.

The reagents were of analytical grade, except HPLC reagents which were chromatographic grade. Ultrapure water was obtained from a Milli-Q System (Millipore Corp., Milford, MA, USA). The mobile phases were filtered through HAWP and HVWP membranes (47 mm diameter and $0.45\ \mu\text{m}$ pore size, Millipore Corp., Milford, MA, USA), used for aqueous and organic solvents, respectively.

2.3. Methods of analysis

2.3.1. Moisture content

The moisture content was determined according to AOAC (1995). It was used to calculate and express bioactive amine levels on a dry weight basis.

2.3.2. Bioactive amines

The amines were determined according to Gloria et al. (2005). They were extracted from the samples with 5% trichloroacetic acid. Three grams of the corn samples were used after grinding and homogenisation. The samples were mixed for 5 min on a shaker at 250 rpm and centrifuged at 8422 g for 20 min at 4°C . This step was repeated two more times. The supernatants were mixed, filtered through qualitative filter paper and through a HAWP

membrane (13 mm diameter and $0.45\ \mu\text{m}$ pore size, Millipore Corp., Milford, MA, USA) and used for analysis.

The amines were separated by ion pair HPLC and quantified after post column derivatization with *o*-phthalaldehyde by means of a spectrofluorimetric detector at 340 nm excitation and 450 nm emission. The column and pre-column used were $\mu\text{Bondapak}^{\text{®}}\text{C18}$ $10\ \mu\text{m}$ ($3.9 \times 300\ \text{mm}$) and $\mu\text{Bondapak}^{\text{®}}$ (Waters Milford, MA, USA), respectively. Two mobile phases were used in a gradient elution: 0.2 mol/L sodium acetate buffer (pH 4.9) with 15 mmol/L sodium octanosulphonate and acetonitrile at a flow rate of 0.8 mL/min.

The identification of the amines was based on comparison of retention times with those in standard solution. The levels of amines in the samples were determined by interpolation from external calibration curves constructed with standard solutions of the ten bioactive amines ($r^2 \geq 0.9696$).

2.4. Statistical analysis

The results were submitted to analysis of variance and the means were compared by the Student's *t* test at 5% probability.

3. Results and discussion

3.1. Moisture content of the different corn products

The moisture content varied significantly among corn products. Higher mean levels were found in canned (78.3 g/100 g) and fresh (74.3 g/100 g) sweet corn. Germinated corn had mean moisture content of 70.9 g/100 g. The dried corn had moisture contents of 11.3–12.7 g/100 g. These values are similar to those reported in the literature (Barbour, Farran, Usayran, & Daghir, 2008; Lupatini, Maccari, Zanette, Piacentini, & Neumann, 2004). Due to the significant differences observed on the moisture contents of the corn products investigated, the levels of amines were calculated and compared on a dry weight basis.

3.2. Profile of free bioactive amines in fresh, canned and dried corn

The profiles of amines in the fresh, canned and dried corn are indicated in Table 1 and Fig. 1. Among the ten amines investigated, spermidine, spermine and putrescine were present in every product analyzed, whereas serotonin, tyramine and tryptamine were not detected in any of the samples. Cadaverine, phenylethylamine, histamine and agmatine were present in different corn products. Cadaverine and phenylethylamine were not detected in canned corn. Histamine was detected only in fresh corn. Agmatine was only quantified in dried corn.

Table 1

Mean levels of free bioactive amines in fresh, canned and dried sweet corn (*Zea mays*).

Amines	Mean levels ^a (mg/100 g dwb)/sweet corn		
	Fresh	Canned	Dried
Spermidine	9.18 ± 0.12^x	10.5 ± 0.42^x	1.90 ± 0.09^y
Spermine	0.94 ± 0.08^z	1.70 ± 0.07^y	2.79 ± 0.16^x
Putrescine	3.40 ± 0.16^y	29.8 ± 2.58^x	0.51 ± 0.02^z
Cadaverine	0.52 ± 0.03^x	nd ^z	0.11 ± 0.01^y
Histamine	0.13 ± 0.00^x	nd ^y	nd ^y
Agmatine	nd ^y	nd ^y	0.32 ± 0.01^x
Phenylethylamine	0.54 ± 0.03^x	nd ^y	0.51 ± 0.08^x

^a $n = 9$ lots analyzed in triplicate; dwb = dry weight basis; nd = not detected; detection limit: 0.1 $\mu\text{g/mL}$; quantification limit: 0.04 mg/100 g; means (\pm standard deviations) with different letters in the same line (^{x,y,z}) are significantly different (Student's *t* test, $p \leq 0.05$).

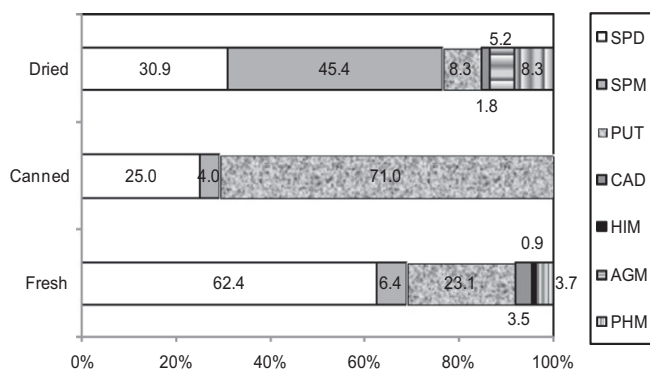


Fig. 1. Percent contribution of each amine to total amines levels in fresh, canned and dried corn (*Zea mays* L.). (SPD – spermidine, SPM – spermine, PUT – putrescine, CAD – cadaverine, HIM – histamine, AGM – agmatine, PHM – phenylethylamine).

The presence of spermidine and spermine was expected in corn products as polyamines are naturally present in vegetables. Putrescine was also expected at low levels as it is an intermediate in the synthesis of spermidine and spermine (Bardócz, 1995; Gloria, 2005). The presence of spermidine and putrescine in fresh and canned corn was reported by Okamoto, Sugi, Koizumi, Yanagida, and Udaka (1997) and Zoumas-Morse et al. (2007); however spermine was not investigated by these authors. Cao, Hua, Zhu, Hua, and Knapp (2010) investigated and detected spermine, spermidine and putrescine in dried corn.

Altogether, spermidine and spermine contributed with more than 67% of the total amines levels in fresh and dried corn, whereas it represented only 29% in the canned corn (Fig. 1). Putrescine was the prevalent amine in canned corn (71% of total levels). Cadaverine, histamine, agmatine and phenylethylamine represented less than 15% of the total amine levels in fresh and dried corn.

Spermidine was the amine which contributed the most to total levels in fresh sweet corn (62.4%). It was followed by putrescine (23.1%), spermine (6.4%), phenylethylamine (3.7%), cadaverine (3.5%) and histamine (0.9%). In canned corn, putrescine contributed the most to total amine levels (71%), followed by spermidine (25%) and by spermine (4%). Therefore, the profile of amines in sweet corn seems to be significantly affected by the canning process. Dried corn contained mostly spermine (45.4%) followed by spermidine (30.9%), putrescine and phenylethylamine (8.3%), agmatine (5.2%) and cadaverine (1.8%).

Based on these results, the profile of free bioactive amines varied significantly among the corn products analyzed. The differences are probably associated with corn cultivars, cultivation practices, and processing steps as described by Shalaby (2000), Liang and Lur (2002), Gloria (2005) and Frías et al. (2007).

3.3. Levels of free bioactive amines in the different products

The total concentration of amines on a dry weight basis ranged from 10.9 to 17.1 mg/100 g in fresh corn, from 14.7 to 79.7 mg/100 g in canned corn, and from 5.0 to 6.9 mg/100 g in dried corn. The mean levels were 14.7, 42.0 and 6.14 mg/100 g, respectively (Fig. 2). The total amine concentration was significantly higher ($p < 0.05$) in canned (42 mg/100 g) compared to fresh (14.7 mg/100 g) and dried corn (6.1 mg/100 g).

According to Fig. 2, the polyamines spermidine and spermine contributed with 70% of the total amines levels in fresh and dried corn; however, in canned corn the polyamines represented only 30% of the amines.

The levels of putrescine in canned corn varied among samples of different brands and among lots of the same brand (data not

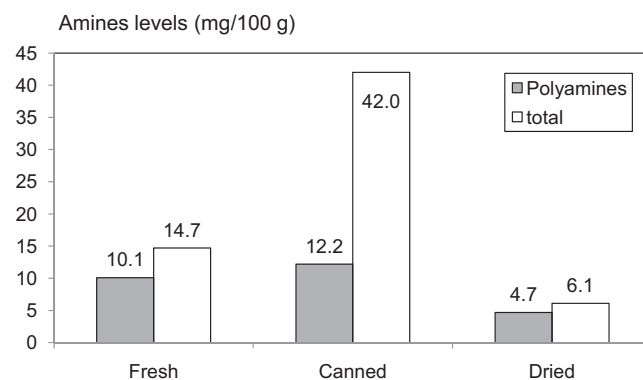


Fig. 2. Polyamines and total bioactive amines levels in fresh, canned and dried corn (*Zea mays* L.).

shown). The highest concentrations of putrescine correlated significantly with storage life – the longer the storage period prior to analysis, the higher the concentration. Studies performed by Shalaby (2000) and Cirilo et al. (2003) indicated that heating and cooking can affect amines levels. Furthermore, putrefactive amines such as putrescine usually increase during storage of food products.

Different levels of amines in corn products were reported in the literature. Okamoto et al. (1997) found higher concentrations of putrescine and spermidine in fresh corn. Zoumas-Morse et al. (2007) reported lower spermidine and putrescine levels in fresh and canned corn. The differences observed on the profile and levels of amines may be related to several factors such as cultivars, cultivation practices, water stress, harvest time, grain maturity, types of processing and storage time (Felix & Harr, 1987; Frías et al., 2007; Liang & Lur, 2002).

3.4. Influence of germination on the profile and levels of amines

The changes which occurred on the levels of amines during germination of corn are indicated in Table 2. During germination, there was a 3–6-fold increase on spermidine and spermine, and a 57-fold increase on putrescine levels. Therefore, during germination, there is a significant increase on the levels of the polyamines and putrescine. This result indicates the higher quality of the germinated compared to regular corn with respect to polyamines.

No significant change was observed on the levels of agmatine on germinated corn compared to regular corn; however, the levels of cadaverine and phenylethylamine were lower whereas the levels of histamine were higher compared to regular corn. The increased histamine levels could be associated with its protective effect against predators during germination (Gloria, 2005).

Table 2

Mean levels of free bioactive amines in corn seeds before and after five days of germination (*Zea mays*).

Amines	Mean levels ^a (mg/100 g dwb)/corn seeds	
	Before germination	After germination
Spermidine	2.00 ± 0.08 ^y	6.89 ± 1.02 ^x
Spermine	1.07 ± 0.09 ^y	5.95 ± 1.1 ^x
Putrescine	0.05 ± 0.00 ^y	2.83 ± 0.23 ^x
Histamine	0.24 ± 0.01 ^y	0.39 ± 0.00 ^x
Agmatine	0.12 ± 0.01 ^y	0.31 ± 0.17 ^x

^a $n = 9$ lots analyzed in triplicate; dwb = dry weight basis; means (\pm standard deviations) with different letters in the same line (^{x,y}) are significantly different (Student's t test, $p \leq 0.05$).

Furthermore, the lower cadaverine and phenylethylamine levels could be associated with their roles in elongation and production of indole, respectively.

In germinated corn (13th day of germination), Boget, Torné, Willadino, and Santos (1995) found lower concentrations of spermidine, spermine and putrescine compared to our results. It would be interesting to investigate the reasons for such difference, whether it is related to the cultivar used or whether the increase observed on the 5th germination day could be followed by decreased levels as germination goes on. However, Gloria et al. (2005) reported results similar to the ones described in this study for germinated soybean: during germination, there was a significant increase on total amine levels (6.6-fold increase in the first 48–72 h), followed by a decrease at 96 h. The significantly higher levels of spermidine, spermine, and putrescine observed at 48–72 h suggested that this was the period with the greatest cellular replication and growth.

According to Felix and Harr (1987), after germination of 30 different plant varieties, including corn (*Zea mays* L.) and soybean (*Glycine max*), there was a sharp increase in the concentrations of polyamines in the cotyledons and endosperm, while cadaverine and putrescine showed variations in total concentrations depending on the type of plant and developing stage.

Frias et al. (2007) evaluated alfalfa and fenugreek sprouts obtained by three different germination conditions (temperature and presence of light) and observed an increase in total bioactive amines content. The levels of amines increased significantly during germination of alfalfa, whereas only spermine levels increased in fenugreek.

Based on the results obtained and also from literature data, the levels of polyamines increase and then decrease during sprouting. However, further studies are needed to determine the time of germination required to achieve maximal polyamines content.

3.5. Profile and levels of amines in different parts of the corn

In order to determine if the profile of free amines in the embryo was different from the endosperm, the two parts were separated and analyzed individually. Canned corn was used for this purpose. Higher concentrations of spermine were detected in the embryo (Table 3), whereas the concentrations of putrescine and spermidine were not significantly different ($p > 0.05$). Therefore, the embryo of the corn had a higher concentration of spermine compared to the endosperm.

For dietary purposes, when a higher concentration of spermine is desired, the embryo of the corn could be used. On the contrary, when reduced levels of spermine are needed, the endosperm could be used. Studies are needed to optimise a process for embryo removal from the corn.

Table 3

Mean levels of free bioactive amines in the embryo and in the endosperm of canned corn (*Zea mays*).

Amines	Levels ^a (mg/100 g dwb)/corn	
	Embryo	Endosperm
Spermidine	5.80 ± 0.46 ^x	4.74 ± 0.38 ^x
Spermine	1.03 ± 0.09 ^x	0.66 ± 0.08 ^y
Putrescine	14.3 ± 2.40 ^x	15.5 ± 2.76 ^x
Total	21.1 ± 0.98 ^x	20.9 ± 1.07 ^x

dwb = dry weight basis.

Means (± standard deviations) with different letters (x,y) in the same line are significantly different (Student's *t* test, $p \leq 0.05$).

^a $n = 3$ lots analyzed in triplicate.

4. Conclusion

Corn was observed to be a significant source of polyamines. Fresh sweet corn contained mainly spermidine followed by putrescine. Spermine, cadaverine, phenylethylamine, histamine and agmatine were also present at lower levels. The profile and levels of amines differed significantly in canned and dried corn compared to fresh corn. Putrescine was the prevalent amine in canned corn whereas spermine was prevalent in dried corn. During germination of corn for 5 days, there was a significant increase on the levels of spermidine, spermine and putrescine. The embryo of the corn contained higher spermine levels compared to the endosperm. Based on these results, corn can be a significant source of polyamines in the diet. The different types of corn products available in the market could be used to attend different dietary and nutritional needs.

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